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QUALITY OF FALSE ACACIA HONEY FROM RASINA DISTRICT IN SERBIA

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Abstract

Honey is made from nectar of flowering plants or honeydew collected by bees which add their own enzymes and store it in their nests for maturation. Honey is a sweet tasting, thick and viscous product which contains various sugars (glucose, fructose sucrose and other polysaccharides), proteins, amino acids, enzymes, organic acids, mineral matter, etc.. Honey is a natural resource of nutrients that are important for human wellbeing. Therefore, it presents an excellent food, essential for daily human consumption.

In this paper, the quality of false acacia honey from the territory of Rasina districts, Republic of Serbia, was studied. Honey was sampled from five locations from three beekeepers and tested using the standard methods for chemical analyses. The organoleptic characteristics, chemical quality indicators, protein, macro- and micro elements and heavy metals were determined.

It was found that there were differences in the quality of honey, primarily depending on the selected sites from which the honey was sampled. There were also differences within a site, depending on the apiary from which honey is taken. The differences were reflected in the color and consistence and, also, in the chemical indicators. The presence of heavy metals was within the allowed limits.

Key words: *False acacia honey, quality, macroelements, microelements, heavy metals*.

1. Introduction

Honey is sweet, thick, crystallized, viscous product that honey bees produce from the nectar of flowering plants or secretions from live parts of plants (conifers and angiosperms). Honey bees collect this matter, add their own specific substances, transform and store in the honeycomb cells to mature (Sl. list SCG 45/03 [1]). Honey is a natural sweet substance produced by honey bees from the nectar of plants or secretions of living parts of plants, or excretion products of insects which live in the plants, that bees collect, transform in the comb with the addition of its specific substances, dehydrate, store in honeycomb to ripen and mature (Codex Alimentarius [2]). Honey is a high-quality food which quality is determined on the content of carbohydrates, water, protein, minerals, pH, vitamins, organic acids, the presence of HMF, the enzyme activity (Codex Alimentarius [2], Bogdanov [3]).

Natural honey is the most complete and highest quality food both for the honey bee and the man. In addition to carbohydrate components, honey contains many other substances that are important in human nutrition. This is primarily related to protein (Christov and Silitskaya [4]), acids (Nelson and Mottern [5]), mineral matter, i.e. macro- and micro elements and, in small but measurable quantity, vitamins (Haydak *et al.* [6], and Kitzes *et al.* [7]), enzymes (White [8]) and other substances that can be found in foods (Arlin [9]). When it comes to the honey bee nutrition, they require carbohydrates which is fully meet by the consumption of honey. Other nutrients (primarily proteins, minerals, vitamins, etc.) are far less present in honey, and the need for these is met by the consumption of the pollen (Adekanmbi and Ogundipe [10]).

Quality control of honey is very important to protect consumers, but also to improve the method of production, processing and use of honey. Consumers expect their information on the quality and origin of honey to be available in the market, based on the quality indicators marked on the label, which are confirmed by analyzes (Council Directive 2001/110/EC [11]). Considering the fact that lately there is an increasing demand for acacia honey from Serbia,

we wanted to determine whether and how it meets the qualitative criteria that are set in our country. In addition, we compared the quality of honey from this region to the quality of honey obtained from this part of Europe (Balkan Peninsula).

2. Materials and methods

Acacia honey samples, analyzed in this study, were collected in 6 municipalities of Rasina region (Krusevac (K), Aleksandrovac (A), Brus (B), Ćićevac (C) and Trstenik (T)). In each municipality, the samples were taken from the three sites. Only the honey produced in 2010 was analyzed. In order to define the quality of acacia honey, certain methods were applied as designated in Sl. list SCG 45/03 [1].

The following chemical parameters were determined: the amount of ash, acidity, diastase activity, reducing and total sugars. In addition to macroelements (nitrogen, phosphorus and potassium), microelements were determined: sodium, calcium, magnesium. Ash was determined by heating a sample of honey in a water bath until most of water evaporates. After that the sample was placed in the sand bath until carbonization. The remainder is then seared in oven, at temperature of 600 °C to constant weight. Before measurement the sample was cooled. The acidity of honey is determined by titration with 0.1 mol/L sodium hydroxide, in the presence of phenolphthalein solution, until the appearance of light pink color. Diastase activity was determined by hydrolysis of 1% starch solution with enzyme from 1 g of honey during one hour at a temperature of 40 °C. Spectrophotometer reading was performed at 660 nm. Reducing sugars were determined by titration, based on the reduction Fehling solution with reducing sugars from honey solution using methylene blue as indicator. Sucrose was determined by the hydrolysis of sucrose, reduction of Fehling solution by titration with reduced sugars from the honey hydrolyzate with methylene blue as indicator. The amount of water in honey was determined using a refractometer at a temperature of 21 °C. Total nitrogen was determined by micro-Kjeldahl method, and other macro- and microelements were determined by AAS (AAS-Perkin Elmer 1100 B USA). Each sample was done in three replicates.

3. Results and Discussion

It is known that the composition and properties of honey vary according to regional and climatic factors under which honey plants grow, the botanical origin of nectar, the length of honey ripening in the hive, storage conditions and other factors (Grujić and Popovic-Raljić [12]). There are many papers where the authors gave a review of the chemical composition and physical properties of honey of different botanical and geographical origin (Thrasyvoulou and Manikis [13], Golob and Plestenjak [14], Marghitas *et al.* [15], Jevtić *et al.* [16], Nedić *et al.* [17], Arsić et al [18], etc).

The amount of ash in the analyzed honeys was average 1.71 gkg^{-1}, the highest value was in the sample from Brus, B-1 (5.53 gkg^{-1}), and minimum was 0.16 gkg^{-1} in the sample from Aleksandrovac (Table 1). It was noted that the most uniform amount of ash was in honey from Kruševac, while in the other location value for this parameter is quite different. According to Sl. list SCG 45/03 [1], the amount of ash in acacia honey can range up to 0.5%, and in some honeys and up to 1%. All analyzed samples were eligible by standard except for one sample from Brus (B-1) which slightly exceeds the limit. Golob and Plestenjak [14] reported that the amount of ash in the acacia honey from Slovenia ranged from 0.3 - 13 gkg^{-1}. The amount of ash in different unifloral honeys in Greece ranged from 1-12 g kg^{-1} (Thrasyvoulou and Manikis [13]).

According to the standard, the amount of water in honey ranges between 16-20%. Honeys which contain more than 20% of water are not mature enough (they were held briefly in the hive), and among them there is a risk of fermentation. Honeys which contain less than 16% water are also faulty matured, meaning that they were probably heated to remove excess water. These honeys tend to have reduced amount of enzymes and increased amount of HMF. The resulting average value of 18.12% and also variation from 17 to 18.7% of water in the analyzed samples show that honey is well matured and that it meets the criteria specified by the standard. Most authors agree in their researches that naturally matured honey from the hive contains between 16-18% of water (Golob and Plestenjak [14], Nedić *et al.* [17], Marghitas *et al.* [15], etc.).

Acids that have been identified in honey as stated by Crane [19] are: gluconic, acetic, butyric, citric, formic, maleic, malic, oxalic, lactic and pyroglutamic acid. Gluconic acid is dominant, and besides listed acids, there are also inorganic acids, such are phosphoric and hydrochloric acid. The acidity of the tested acacia honey ranged from 10.04 - 20.07 $mmolkg^{-1}$ with an average value 13.87 $mmolkg^{-1}$. The results showed that all of the samples meet the criteria stated by Sl. list SCG 45/03 from 2003 [1], which allows up to 40 $mmolkg^{-1}$ of acid. Quantity of acids in honey from Slovenia ranged from 20.15 to 30.99 $mmolkg^{-1}$ (Golob and Plestenjak [14]), while in acacia honey from Transylvania there was something lower acid content (14.3 $mmolkg^{-1}$), (Marghitas *et al.* [15]). Popa *et al.* [20] found 12-14 $mmolkg^{-1}$ of acid in acacia honey from Transylvania,

while in flower honey (polifloral) was significantly more acid from 20.6 - 55.6 mmolkg^{-1}.

The amount of enzyme that bees add to nectar shows us that the naturalness of honey, meaning whether the honey was produced naturally or has been a forgery. According to the standard, diastase activity must not be less than 8. All of the tested samples have a higher value than that determined by the standard. One sample (C-1) has a much higher diastase number than others, which can point that there is other impurities in it. Diastase activity may be of 8.6 with honey from orange up to 51 for honey from thyme (Thrasyvoulou and Manikis [13]).

The results are similar to that obtained in the research and Plestenjak Golob [14], and slightly lower than the results of Oddo *et al.* [22] and Vorolova and Čelechovska [21]. Carbohydrates or sugars are the main component of honey and they make 70-75% of the total content of honey. Carbohydrates can make over 95% of dry matter in honey, while the other compounds are accounted for less than 5%. Standard requires that the amount of reducing sugars in honey (glucose and fructose) should be above 65% except for forest honey which must have more than 60%. The amount of sucrose in acacia honey can be up to 10%. All of the samples had satisfactory amount of reducing sugars, except for one sample that was on the verge (T-3), and intervals of variation ranged from 64.66 to 75.19%. Sucrose content was also within the standard limits and ranged from 1.80 to 9.63%.

Table 1. Chemical indicators of quality of honey from Rasina region in Serbia

Sample	Ash gkg^{-1}	Water 21^{0}C (%)	Acidity mmolkg^{-1}	Diastase activity	Total sugar (%)	Reducing sugar (%)	Sucrose (%)
K-1	0,80	18,2	12,05	9,81	80,30	72,78	7,52
K-2	0,27	18,0	14,05	8,96	81,80	75,19	6,61
K-3	0,22	18,4	17,06	9,27	81,80	69,17	9,63
A-1	4,11	17,0	10,04	11,84	80,09	71,58	8,12
A-2	0,16	17,5	10,04	9,36	74,28	67,10	7,18
A-3	0,16	18,7	20,07	8,59	68,57	66,77	1,80
B-1	5,53	18,8	14,05	10,28	69,74	66,16	3,31
B-2	2,64	18,8	16,06	10,01	69,47	66,16	3,31
B-3	0,75	18,6	16,06	9,53	68,87	66,77	2,10
C-1	1,13	18,7	14,05	9,87	77,59	72,78	4,81
C-2	2,43	17,9	17,06	13,28	80,00	72,18	7,82
C-3	0,51	17,0	13,05	9,25	73,38	66,46	6,92
T-1	3,89	18,1	12,05	9,90	74,29	71,28	3,01
T-2	1,38	18,7	9,03	9,08	73,08	70,07	3,01
T-3	1,74	17,4	12,05	10,87	73,68	64,66	9,02
Average	**1,71**	**18,12**	**13,78**	**9,99**	**75,13**	**69,27**	**5,61**
SD	**1,67**	**0,64**	**3,07**	**1,22**	**4,81**	**3,21**	**2,66**

Of other chemical indicators, the presence of macro- (N, P, K, Na, and Ca) and microelements (Mg, Zn, Fe and Mn) was studied, and the results were shown in Table 2. For the normal brood development, honey bees use large quantities of nitrogen which it gets from the pollen which is the most important source of protein. Proteins are present in honey mainly due to pollen in honey.

Also, nitrogen in honey may be present in the form of amines, amides and less in the form of amino acids. Average value was 360 mgkg^{-1} (0,036%). The highest value was in the honey from Brus (770 mgkg^{-1}), and the lowest in the ample from Trstenik (150 mgkg^{-1}). Potassium is a substance that has the highest presence of all investigated minerals in acacia honey. The amount of potassium was average of 141.81 mgkg^{-1}, and varied from 62.8 - 212.0 mgkg^{-1}. These values and even greater were determined by the majority of authors who dealt with this issue. Mladenović *et al.* [23] found that acacia honey has 228mgkg^{-1} of potassium, and basswood honey has much more of this element 1020mgkg^{-1}. In his research Yilmaz and Yavuz [24] found that honey has 296mgkg^{-1} op potassium a lot more than the results obtained in our research.

White [25] stated that the permitted level of sodium is in between 6-40mgkg^{-1}. The resulting amount of sodium in our research is an average of 47.75 mgkg^{-1} with an interval varying from 23.1 to 81.3 mgkg^{-1} which is higher than values obtained in the research of Thrasyvoulou and Manikis [13]. Obtained results are different from the results of Kulincević [26] in which the light honeys have concentration of 251 mgkg^{-1} of sodium, and dark honeys only 23 mgkg^{-1}.

The average amount of calcium obtained in this study was 40.31 mgkg^{-1} and varied from 29 to 54.6 mgkg^{-1}. Permissible amount of calcium by White [25] is 40-300mgkg^{-1}. Yilmaz and Yavuz [24] analyzed 30 honey samples and found average concentrations of calcium 52mgkg^{-1}, Chakir *et al.* [29] suggest that this element in the honey from Morocco varies from 20 to 196 mgkg^{-1}.

Table 2. Content of macro- and microelements in honey from Rasina region in Serbia

Sample	N, mgkg^{-1}	P mgkg^{-1}	K mgkg^{-1}	Na mgkg^{-1}	Ca mgkg^{-1}	Mg mgkg^{-1}	Zn mgkg^{-1}	Fe mgkg^{-1}	Mn mgkg^{-1}
K-1	420	0,008	210,0	70,1	29,1	6,83	2,56	2,11	0,52
K-2	280	0,009	212,0	68,2	54,6	5,28	3,28	0,58	0,33
K-3	460	0,012	206,1	81,3	33,1	8,45	1,41	3,45	0,62
A-1	540	0,008	168,5	58,3	52,6	3,78	3,28	2,53	0,11
A-2	520	0,008	106,0	37,4	29,0	4,29	6,51	3,22	0
A-3	410	0,008	137,1	39,2	41,8	6,81	3,23	1,62	0,09
B-1	250	0,008	156,0	47,6	43,0	9,24	2,58	1,89	0,28
B-2	770	0,011	150,5	50,3	41,9	1,23	2,86	3,05	0,62
B-3	440	0,004	150,2	36,0	33,1	6,85	1,92	4,21	0,59
C-1	200	0,008	112,3	32,3	41,2	7,44	2,89	2,22	0,33
C-2	220	0,008	150,3	23,1	43,5	8,56	3,14	3,01	0,45
C-3	250	0,008	100,0	44,9	29,2	6,49	2,09	1,63	0,27
T-1	290	0,008	131,2	45,0	41,4	9,81	1,53	1,92	0,61
T-2	150	0,000	75,0	45,1	43,9	9,11	3,33	2,55	0,21
T-3	200	0,009	62,8	37,5	47,2	11,23	2,84	2,34	0,28
Average	**360,0**	**0,0078**	**141,87**	**47,75**	**40,31**	**7,03**	**2,90**	**2,42**	**0,35**
SD	**168,82**	**0,0028**	**45,97**	**15,71**	**8,109**	**2,59**	**1,18**	**0,89**	**0,21**

The amount of magnesium is quite different for the individual samples and the localities from which samples were taken. Most magnesium is obtained in the honey from Trstenik (T-3), and least in the honey from Brus (B-2). Mladenović *et al.* [23] reported the amount of magnesium 4-77 $mgkg^{-1}$, and Chakir *et al.* [29] found that manganese is present an average of 26.50 $mgkg^{-1}$. Kulincević [26] found that light honeys contain up to 40 $mgkg^{-1}$, and dark honeys contain up to 132 $mgkg^{-1}$ of magnesium. Thrasyvoulou and Manikis [13] state that acacia honey from Romania has a 5.70 $mgkg^{-1}$ of magnesium which is slightly lower value than in our study. The amount of zinc in the analyzed honey ranged from 1.51 to 6.41 $mgkg^{-1}$. Any increase above the 5.6 $mgkg^{-1}$ indicates that honey is kept in inadequate packaging or was centrifuged in a galvanized centrifuge. Similar results for this element are given by many authors (Mladenović *et al.*, [23], Thrasyvoulou and Manikis [13], Yilmaz and Yavuz [24], Chakir *et al.* [29]). Iron was, in all but one sample from Kruševac (K-2), very evenly represented in the analyzed honeys from Rasina region. On average was obtained 2.42 $mgkg^{-1}$. As stated by Janković [27], iron concentration in honey is in the range of 0.1 to 2.05 $mgkg^{-1}$. However some authors (Marinova *et al.* [28]) reported a much larger range of variation of the concentration of this element (4.1 to 20.2 $mgkg^{-1}$). Most iron content in the honey from this region was found in the researches of Kulincević [26] in dark honeys 37$mgkg^{-1}$.

3. Conclusions

- Analyzed honey from Rasina region in Serbia meets all the requirements of the national standard (Sl. list SCG 45/03, 2003) and EU Directives (Council Directive 2001/110/EC).
- When it comes to reducing sugars, it was determined that there was one sample that did not meet standards because it showed the 64.66% of reducing sugars, while the standard requires 65%.
- Considering macro- and microelements, there are variations between honeys taken from different municipalities and between honeys from the same municipality, but of different beekeepers.

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